STUDIES ON THE FORMATION, FLOW AND OUTFLOW OF AQUEOUS HUMOR

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Studies on the Formation, Flow and Outflow of Aqueous Humor

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The present article comprises a summary of earlier investigations which have been reported in the following papers:

I. L. Berggren: On the appearance of fluorescent dyes in the aqueous humor after intravenous injection. Am. J. Ophthalm. 42, part I, 595 (1956).

II. L. Berggren, F. Vrabec: Demonstration of a coating substance in the trabecular meshwork of the eye: and its decrease after perfusion experiments with different kinds of hyaluronidase. Am. J. Ophthalm. 44, 200 (1957).

III: L. Berggren: A histochemical study of the anterior segment of the eye in gold id (Idus Idus L.), chicken, rabbit and man with special regard to the angle of the anterior chamber. Acta Soc. Med. Upsal. 62, 157 (1957).

IV. L. Berggren: Autologous transplants of chamber angle tissue into the anterior chamber in albino rabbits. Observations on vascularization and content of acid mucopolysaccharides. Acta Soc. Med. Upsal. 64, 111 (1959).

V. L. Berggren: Further observations on the appearance of fluorescein in the rabbit eye after intravenous injection. Acta Ophthalm. 37, 215 (1959).

VI. L. Berggren: Demonstration of metachromasia and its distribution in the anterior chamber of the tawny owl (Strix Aluco) using a new printing technique on plaster slides. Acta Anat. 38, 140 (1959).

VII. L. Berggren: Intracellular potential measurements from the ciliary processes of the rabbit eye in vivo and in vitro. Acta Physiol. Scand. in press (1959).

In the following these articles will be referred to using the Roman numerals given above.

The ophthalmological interest in the physiology of the intraocular pressure is partly derived from the challenge to therapy that glaucoma still offers. The methods of treatment, the benefits of which we can follow but still do not entirely understand, will even at their best remain palliative as long as there is so much imperfect knowledge of the physiological basis of the chronically increased intraocular pressure; the formation of aqueous—its rate of flow in the anterior parts of the eye—and its outflow through a resistant barrier.

Formation

The old conception, that aqueous is secreted from the ciliary epithelium is now re-accepted in a modified form. Initially, this was a purely anatomic conclusion based on the observations, that ciliary epithelial cells resemble a secreting epithelium and consequently were ascribed a glandular function.—The similarity between aqueous and protein free plasma led to the theories of aqueous as a dialysate or ultrafiltrate of plasma. Closer chemical analysis of the components in aqueous and plasma, and the rate of accumulation and turnover of various substances exposed the shortcomings of this theory, and the modern conception of aqueous humor formation stipulates that at least some components must be secreted into the posterior chamber and most probably by the ciliary epithelial layers. In the mathematical treatment of the aqueous humor data, however, no notice was taken of electrical gradients between aqueous and plasma and a more refined evaluation of the passage of ions into the posterior chamber must take potential differences across the ciliary epithelial layers into account. Little is known about such potential differences. Lehmann and Meesmann (1) put KCl-calomel electrodes into the jugular vein as well as into the anterior chamber of rabbits and measured the potential difference. Friedenwald and Stiehler (2) demonstrated a difference in oxidation-reduction potential between the ciliary epithelium and the stroma.

However, in the ciliary epithelial layers there are several barriers across which transport must pass. To analyze the transport it seems inevitable to measure the potentials step by step across the different barriers. The only reliable method to attack these problems seemed to be to penetrate the ciliary processes by intracellular electrodes recording the potentials as the electrode advances through the different barriers. This has been attempted in paper VII.

Intracellular Potential Recordings from the Ciliary Processes of the Rabbit Eye in vivo and in vitro. (VII)

To register the potential differences across the different layers of the ciliary processes a microelectrode device was constructed. A micrometer carrying the microelectrode was attached to a heavy stand and ad-

vanced by hand or a motor. Continuous as well as discontinuous movement was used. The potentials were recorded on a strip chart. To record the position of the microelectrode a depth registration impulse was also transferred to the recorder.

In a normal living eye it is impossible to get access to the ciliary processes in the posterior chamber with a microelectrode. In the experiments conducted in vivo these difficulties were avoided by a transplanting operation prior to the microelectrode experiments. An iris-ciliary body strip from one eye was transplanted to the anterior chamber of the opposite eye. In successful cases the transplanted iris portion got attached to the host iris and the ciliary body remained free in the aqueous. After trephining the cornea a microelectrode could be advanced into the ciliary process in vivo.

There were considerable technical difficulties in the in vivo method and the main part of the experiments were performed with an in vitro method.

The anterior parts of a rabbit eye were placed in a physiological buffer and the ciliary processes now freely accessible were penetrated by a microelectrode entering from the free surface of the epithelium.

As the microelectrode advanced two potential levels of different size were recorded. The first potential was about -30 millivolts and the second -60 millivolts. Each potential step was recorded during a distance of about 20 μ. A comparison with the anatomy of the processes made it highly probable that the first potential originated from the unpigmented ciliary layer and the second potential from the pigmented layer.

The stromal potential, that is, the potential difference between the stroma and the outer medium was about -5 millivolts both in the experiments conducted in vitro and in vivo.

An analysis of the exact nature of the transport across the ciliary epithelial layers requires further investigations. The developed technique looks promising in that respect. By alternation of the composition of the bath solution it might be determined what ions are indispensable to maintain the potential differences. The introduction of various pharmacological agents known to affect secretion processes could show the effect of these drugs on the potential in the ciliary epithelial layers. Such procedures might considerably advance our knowledge of the transport mechanisms in the ciliary processes.

It was shown in the present investigation that the membrane potential disappeared in a potassium free solution or in the presence of 1 mg/ml potassium cyanide.

Flow

The rate of flow of aqueous can be calculated from time-concentration curves of different substances in the aqueous. This is a time-con-

suming method and an assumption must be made that the eyes are in steady state conditions during the whole experimental period. The method of Goldmann (3) based on the accumulation of fluorescein in the anterior chamber could be used clinically since it avoided punctures of the anterior chamber. The great role determinations of rate of flow can play in physiological and pathophysiological connections makes a quick, reliable and easy method most desirable. In certain situations the need has been filled by tonography. Another method is founded on time determinations of the appearance of fluorescein into the anterior chamber after an intravenous injection.

On the Appearance of Fluorescent Dyes in the Aqueous Humor after Intravenous Injection. (I)

If one observes an eye after an intravenous injection of fluorescein a green cloud will appear in the pupil after a certain time interval. This interval is called the appearance time and it has been suggested that such measurements may be used in the study of changes in the rate of production of aqueous humor. The validity of the measurement, however, rests on certain assumptions. Firstly, that the green cloud emanating in the pupil is the fluid entering from the posterior chamber. Secondly, that the appearance is determined by the convection to the pupil and not by the permeation of the dye into the aqueous. While appearance time should be a function of the dose when small doses are used, and diffusion is the rate-limiting factor, the quantity injected should be of no importance when large enough doses are used, and convection becomes rate-limiting. The appearance of fluorescence in the anterior chamber (a fluorescent Tyndall beam) was determined on pigmented rabbits after an intravenous injection of three fluorescent dyes. The results indicate that at low doses the product of appearance time and dose is constant, suggesting diffusion as the determining factor but at higher doses at a time limit of approximately five minutes the product was no longer constant indicating a new rate-limiting factor, probably convection which influences the time of appearance. Using ultra violet slit lamp microscopy the appearance time (a green cloud in the pupil) was determined for the three fluorescent dyes after a large intravenous injection. The appearance time for the three different dyes were rather similar indicating that large doses of substances with different rates of permeation yield the same appearance time. All these facts support the assumption of appearance time as a valid method in the study of rate of production of aqueous. Other experiments, however, show that factors that influence convection in the anterior chamber such as the outside temperature and the position of the globe also influence the appearance time. Nor could an increase in appearance time be

demonstrated after previous treatment with the carbonic anhydrase inhibitor acetazoleamide. The results indicate that under certain circumstances the appearance time will not be a measurement of convection in the posterior chamber but due to convection in the anterior chamber.

A Further Contribution to the Appearance of Fluorescein in the Eye of the Rabbit after Intravenous Injection. (V)

To study more thoroughly the course of events leading to the appearance of a green cloud in the pupil (the appearance time) after an intravenous fluorescein injection, it would be desirable to be able to follow simultaneously the processes in the posterior and anterior chamber. This is obviously very difficult to do in a living animal and therefore in the present paper a different approach was used. After an intravenous injection of a large dose of fluorescein in different strains of rabbits (albino and pigmented animals), the eyes were frozen in situ at various intervals after injection. The eyes were sectioned to give sagittal eye-halves and the distribution of fluorescein could be studied simultaneously in the anterior and posterior chamber by observation in ultraviolet illumination. The cloud in the pupil was seen to emanate sometimes from the posterior and sometimes from the anterior chamber. Various rabbit strains seemed to differ in this respect. There was less diffusion from the anterior iris vessels in the pigmented rabbits than in the albino rabbits. An appearance time, however, of more than 2 minutes will be influenced by processes in the anterior chamber also in pigmented rabbits.

Selected animal strains might thus be suitable in studies of the rate of production of aqueous with the appearance time method, but determinations at decreased aqueous humor production, for example after treatment with carbonic anhydrase inhibitors, can be unreliable if there are no possibilities to differentiate between flow from the posterior chamber and diffusion and convection in the anterior chamber.

Outflow Resistance

Passage of aqueous from the anterior chamber and out of the eye goes via the trabecular meshwork, the canal of Schlemm and the intra- and episcleral venous plexuses. There has been some controversy about the main site of the resistance to outflow of aqueous but a vast majority of clinical and experimental evidence has placed the main part of the resistance at the level of the trabecular meshwork. The perfusion studies by Bárány (4–7) demonstrating a hyaluronidase-sensitive component

in the trabecular meshwork introduced a new concept to the understanding of the regulation of the aqueous outflow. It was found that if hyaluronidase was added to the perfusion fluid the resistance decreased by about 50% in different species. As to the size of the outflow channels Huggert (8–9) using suspensions of chromium phosphate or bacteria could demonstrate that the particles which could pass through the trabecular meshwork had a maximal size of 1.2–2.4 μ in rabbit eyes and 1.5–4.4 μ in human eyes. This was also confirmed by microradiography (François, Neetens, Collette (10)).

The exact nature of the passage of aqueous through the trabecular meshwork and how this passage is regulated remains an unsolved problem.

The present investigations have mainly been concerned with the hyaluronidase-sensitive barrier in the trabecular meshwork. This barrier which is easily demonstrated by physiological perfusion methods was found to be difficult to demonstrate histologically.

In paper II perfusion experiments were performed. With a replica method there could be shown to be a correlation between a fall in resistance after hyaluronidase and a change in surface structure of the trabecular meshwork. There remained, however, the question of the origin of the hyaluronidase-sensitive substance, if there is a local formation or if the substance is transported to the trabecular meshwork.

In an earlier paper, Bárány, Berggren and Vrabec (11) showed the existence of a hyaluronidase-sensitive substance in the anterior chamber of the tawny owl and it was suggested that the material was produced by the corneal endothelium. This was supported by the findings in paper VI where the distribution of metachromatic material in the anterior chamber of the tawny owl was studied. The kinship previously suggested by Salzmann (12) between the corneal endothelial cells and the cells covering the trabeculae was established in a histochemical investigation, paper III. From the findings that the corneal endothelial cells showed the same enzyme spectrum as the cells covering the trabeculae it was suggested that they had a similar function. In consequence to this the latter were thought to control the hydration of the trabeculae and in changing the pore size also be involved in the passage through the trabecular meshwork.[1] A common secreting function (of a viscous material) is another possibility. The problem of local formation was also treated in a different way. In paper IV it could be demonstrated that there were increasing amounts of hyaluronidase-sensitive acid mucopolysaccharides in non-filtering chamber angles compared with the normal chamber angle. These results were in favour of a local production of hyaluronic acid in the trabecular meshwork.

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[1] Holmberg (to be published (13)) now has shown that it is possible that the flow-limiting pores are in the endothelial lining of the canal of Schlemm.

Demonstration of a Coating Substance in the Trabecular Meshwork of the Eye: and its Decrease after Perfusion Experiments with Different Kinds of Hyaluronidase. (II)

Using a perfusion technique Bárány (4–7) could demonstrate in a variety of animal eyes the existence of a hyaluronidase-sensitive component in the resistance to outflow of aqueous. Any histologic demonstration of the mucoid constituting this barrier was, however, lacking when this paper was published.[1]

In this paper a replica method was used to demonstrate structural changes in the trabecular meshwork. Eyes from cattle, rabbits and sheep were used. One eye served as control eye perfused only with cattle aqueous. The pair eye was perfused with a hyaluronidase solution in cattle aqueous. Replicas of the trabecular meshworks from both eyes were then compared. To improve specificity of the hyaluronidase treatment different kinds of hyaluronidases were used; testicular and bacterial hyaluronidase as well as snake and bee venom.

It could now be demonstrated, that there is a coating substance in the trabecular meshwork of cattle, rabbits and sheep, which were previously perfused with cattle aqueous. Moreover, this coating substance could be seen to be removed in the pair eyes, which had been perfused with different kinds of hyaluronidase solution. Mechanical trauma, which is known to start a process which breaks down the hyaluronidase-sensitive barrier also caused a reduction of this substance. It was further shown that in eyes where the hyaluronidase-sensitive barrier was broken down but the resistance had returned to normal level, there were some structural changes. These changes, possibly indicating swelling of the trabeculae, might be due to a compensating mechanism maintaining the resistance at a normal level.

In order to avoid auto-suggestion in the comparison between control and test eye a special "blind" set up was used. In the results presented here the probability that the right answer was picked by chance was less than 0,001. The morphologic changes in the trabecular meshwork after hyaluronidase treatment were thus established beyond doubt.

Demonstration of Metachromasia and its Distribution in the Anterior Chamber of the Tawny Owl (Strix Aluco) Using a new Printing Technique on Plaster Slides. (VI)

Abelsdorff and Wessely in 1909 (15) demonstrated that the aqueous humor in the owl was highly viscous. These findings became of renewed interest when it was shown that there was a hyaluronidase-sensitive

[1] Later demonstrated by Zimmerman (14).

barrier to the outflow of aqueous. In an earlier paper, Bárány, Berggren and Vrabec (11) could demonstrate that the viscous substance in the tawny owl could be broken down by bacterial hyaluronidase. The viscosity was highest close to the lens and seemed to decrease inwards.

It seemed desirable to know the distribution of this viscous material in the anterior chamber in order to see how circulation of aqueous is possible in the owl eye. Another interesting question was if the material was locally produced or if it was transported to its location.

Since it was impossible to preserve the in vivo arrangement of the anterior chamber contents with normal histologic technique, a new procedure was worked out.

Frozen eye sections of the tawny owl were put onto slides made of plaster. The melting ice was sucked into the plaster slide and a print of the structures in the section was obtained on the surface of the plaster slide. The contents of the anterior chamber were visualized by metachromatic staining of the deposit.

Immediately behind the cornea a broad metachromatic zone of uniform tone and saturation was found. This zone was separated from the lens and iris by a thin non-metachromatic zone peripherally reaching the uveal meshwork. This uneven distribution of metachromatic material in the anterior chamber must be due to the presence of a gel (presumably hyaluronic acid) which does not easily dissolve in aqueous humor.

The results show that there is a possibility for aqueous humor circulation in the owl. Moreover, the localization of acid mucopolysaccharides with a free zone close to the lens indicates a local production by the corneal endothelium.

A Histochemical Study of the Anterior Segment of the Eye in Gold Id (Idus Idus L.), Chicken, Rabbit and Man with Special Regard to the Angle of the Anterior Chamber. (III)

The ontogenetic and phylogenetic development of the eye shows that the trabecular meshwork has passed from a structure consisting of epitheloid cells into one that is rich in connective tissue. Endothelial cells are still abundant, however, even in the human trabecular meshwork. It was already suggested by Salzmann (12) that these endothelial cells represent a continuation of the corneal endothelial cells. This suggestion was never disputed although any direct evidence of a close similarity was lacking. If there is a close anatomical similarity, however, there would be reason to expect that the function of the corneal endothelial cells and the cells covering the trabeculae also would be similar. Experiments with de-epithelialized cornea (Davson 16, Harris 17–19) have shown that the corneal endothelial cells take a major part in the

dehydration of the cornea by means of active transport. In consequence to this the cells covering the trabecular meshwork could be involved in controlling the hydration of the trabeculae and thus by changing the pore size they could influence the resistance to passage through the meshwork. The kinship between the corneal endothelial cells and the cells covering the trabecular meshwork were studied in this paper by histochemical demonstration of various enzyme systems.

The eyes were frozen with isopentane cooled in liquid air (−170°C). Sections from the ice block were cut in a cryostat (−20°C). The enzyme systems studied were non-specific alkaline phosphatases, acid phosphatases, esterases and the succinic dehydrogenase system. Such enzyme systems have been demonstrated in abundance in other parts of the body where active transport occurs.

The presence of considerable amounts of succinic dehydrogenase system and esterases were demonstrated in the cells of the trabecular meshwork (in chicken, rabbit and man) and in the cells of the annular ligament (in the gold id). In the same cells lively nonspecific alkaline phosphatase activity was found in the gold id and chicken but not in rabbit and man and moderate non-specific acid phosphatase activity in all species.

The enzyme spectrum of the endothelial cells covering the inside of the cornea were similar to that of the cells of the trabecular meshwork (respectively annular ligament) in all species.

Autologous Transplants of Chamber Angle Tissue into the Anterior Chamber in Albino Rabbits. Observations on Vascularization and Content of Acid Mucopolysaccarides. (IV)

The existence of hyaluronidase-sensitive material in the trabecular meshwork is established and the resistance fall that can be demonstrated in perfusion experiments after hyaluronidase administration shows that this material is an important component in the resistance to outflow. Are the structures of the meshwork also the site of formation of this material? If this were the case one would expect that under certain conditions as for example pathological processes in the trabecular meshwork leading to impaired filtration the amount of hyaluronidase-sensitive material might be affected and consequently the degree of resistance. Such processes would give rise to an increased intraocular pressure and cause glaucoma. It is, however, not altogether clear that the trabecular meshwork where the hyaluronidase-sensitive material is deposited also is its site of formation since the substance could have been transported from another site by aqueous flow.

To elucidate these problems it would be desirable to study and compare normal chamber angle structures with those with impaired or

abolished passage of aqueous. In the absence of local formation we would not expect any hyaluronidase-sensitive material in non-filtering angles, whereas if there is a local formation we would expect increasing amounts of such substance in the non-filtering chamber angles.

To fulfil the conditions described above, a section of the chamber angle from one eye was transplanted to the anterior chamber of the opposite eye in albino rabbits. In the surviving transplant strip no filtration occurs whereas the chamber angle structures of the host eye have normal filtration. A comparison was made between these structures with different methods for the demonstration of acid mucopolysaccharides. Since there is no specific method yet for a histologic demonstration of hyaluronic acid, several methods were used and their specificity further increased by incubations with different hyaluronidases. No hyaluronidase-sensitive material could be demonstrated in sections from normal chamber angle structures. The transplants on the other hand, that is the non-filtering chamber angles, showed rich amounts of such substances.

The results thus strongly point to a local formation of the hyaluronidase-sensitive material. It seems reasonable to assume that even moderate variations in filtration could give rise to altered amounts of substance and in consequence of this to altered resistance conditions. The origin of a vicious circle would be established where an impaired filtration gives rise to an increased resistance which in turn yields a further impaired filtration. In a clinical glaucoma report Goldmann (20) described similar conditions where he pointed out that if the wound closes after a filtering operation the glaucoma might be in a worse condition than prior to operation.

An interesting accidental finding concerned the vascularization of the transplants. It could be demonstrated by corneal microscopy that growth of new vessels occurred generally into the scleral part of the transplant, seldom into the corneal part and never into the site of the trabecular meshwork. This might be due to the presence of a repellent factor or the lack of an attracting factor in the trabecular meshwork. Isolated vascularization of the trabecular area is rare, but can be found in rubeosis iridis diabetica. The interesting fact that diabetes also is accompanied by a decreased hyaluronic acid production (demonstrated in the skin of rats by Schiller and Dorfman (21, 22)) gave rise to speculations as to whether hyaluronic acid might be a factor in the avascularity of the trabecular meshwork.

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